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Roles of lncRNA in breast cancer

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1. ABSTRACT

Recent systematic genomic studies have revealed a broad spectrum of lncRNAs that are involved in a variety of disease (diseases), including tumor progression, by regulating gene expression at epigenetic, transcriptional and post-transcriptional levels. However, their exact roles of physiological function and the mechanism (mechanisms) of action are yet to be clarified. In breast cancer research, several lncRNAs are identified as tumor driving oncogenic lncRNAs and few are identified as tumor suppressive lncRNAs. They are involved in cell growth, apoptosis, cell migration and invasiveness as well as cancer cell stemness. Therefore, this new class of RNAs may serve as biomarkers for diagnostic and prognostic purpose and also as potential therapeutic targets. This review summarizes the current information about lncRNAs that are particularly involved in breast cancer progression and also discusses the potential translational application of these newly discovered nucleic acids.

2. INTRODUCTION

Recent genomic and bioinformatic studies across the species have revealed that eukaryotic genomes transcribe broad spectrum of RNAs including protein coding mRNAs, short non coding (non-coding) transcripts and long non-coding RNAs (lncRNAs) (1). Among these RNAs, non-coding transcripts are found to be more abundant in human cells. Up to 70% of the human genome is transcribed; however, only 2% of them are translated into protein (2). In the past several years, short non-coding RNAs such as microRNAs, small interfering RNAs and snoRNAs have been extensively studied, while lncRNAs have drawn relatively less attention. However,

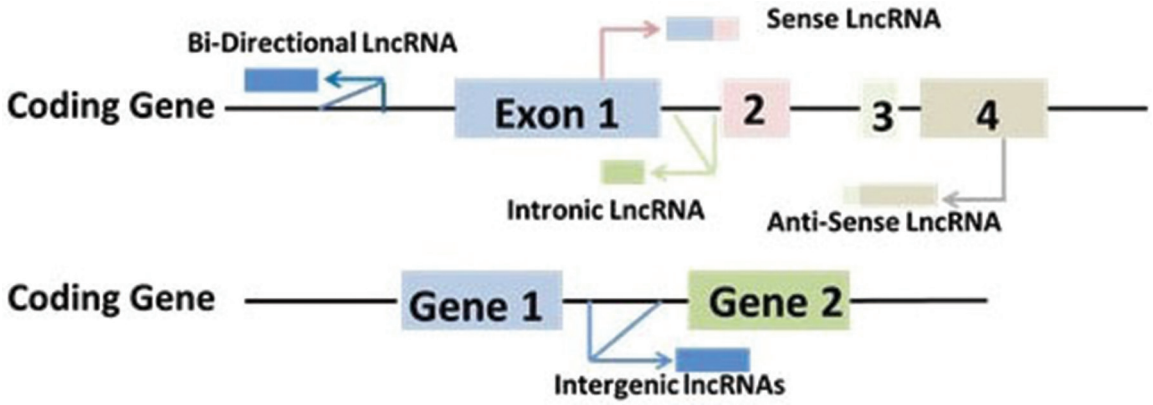

Figure 1. Five categories of lncRNAs.

it has become increasingly apparent that lncRNAs are not simply leaky products of the genome, and many of these RNAs have been experimentally characterized to show their distinct cellular functions. Importantly, many of these transcripts are associated with a variety of human diseases.

LncRNAs can be defined as RNA transcripts longer than 200bp that lack open reading frames. A number of lncRNAs were initially identified through the whole genome tilling array and the next generation sequencing of transcriptome. These studies showed that lncRNAs have complicated structures and intrinsic origins, and therefore, they can no longer be defined just by their length and protein-coding incapability. However, lncRNAs have several common features. The chromatin state of lncRNAs are consistent with protein coding genes, marked by trimethylation of lysine4 of histoneH3 (H3K4me3) at gene promoter and trimethylation of lysine36 of histone H3 (H3K36me3) along the transcribed region (3). Expression of lncRNAs are often regulated by well-known transcription factors, and the ENCODE project reported that there are multiple transcription factors that preferentially regulate lncRNA transcription (4). Like the coding genes, lncRNAs are transcribed by RNA polymerase II and usually spliced by the splicesome. LncRNAs also have a polyA tail as the coding mRNAs (3).

LncRNAs can be defined into five categories depending on their origin in the genome as shown in Figure 1 (5). The first group is **Sense lncRNA.** Sequence of these lncRNAs overlap with one or more exons of another transcript in the same direction. The second category is **Antisense lncRNA.** Sequence of these lncRNAs overlap with one or more exons of another transcript in the opposite direction. The third category is **Bidirectional lncRNA, and** these lncRNAs are initiated in close genomic proximity with a neighboring protein coding transcript on the opposite strand. The fourth group is **Intronic lncRNAs.** These lncRNAs are wholly derived from within an intron of another transcript. Sometimes these may represent pre-mRNA sequence. The last category is **Intergenic lncRNAs**, and these lncRNAs are derived from sequence within the genomic interval between two genes.

3. PHYSIOLOGICAL FUNCTIONS OF LNCRNAS

Recent comprehensive study of the human genome have identified over 8,000 lncRNAs (5). Other studies also reported that there are several thousands of lncRNAs expressed in human and other mammals (3,6). However, many of these transcripts are not conserved in closely related species, and only ~200 lncRNAs are functionally characterized and mechanistically well defined (7). This raises the question that whether all lncRNAs are biochemically functional. Most lncRNAs are expressed in a tissue specific manner, suggesting the potential biological and physiological functions (5). Among the 200 lncRNAs that have been studied so far, many of them showed evidence of functionality *in vitro* or *in vivo*, although only a few were characterized *in vivo*. Judging from the data to date, it is clear that lncRNAs are involved in a wide range of biological and physiological processes and hold distinct functions at each step. Moreover, lncRNAs are important regulators in tumor-suppressor and oncogenic pathways, and recent studies showed that lncRNAs regulate the major cancer-driving pathways at epigenetic, transcriptional and post-transcriptional level as outlined below (3).

3.1. Epigenetic regulation

The expression of genes are often controlled by epigenetic factors such as chromatin-modifying complexes and DNA methyltransferases, and many tumor suppressive genes are found to be inactivated by epigenetic silencing during tumor progression (8, 9). The most well-known function of lncRNAs is related to epigenetic regulation of target genes, especially by their repressive functions. Many lncRNAs, including ANRIL,

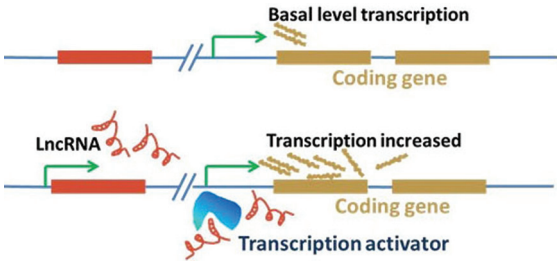

Figure 2. LncRNA enhance transcription. LncRNA transcripts transcribed from ~1kB upstream of coding gene tethers transcription factors to the promoter of the coding gene which enhances the gene expression.

HOTAIR, H19 and XIST, achieve transcription repression by coupling with histone-modifying or chromatin-remodeling proteins (10). LncRNAs are believed to be operated in both cis and trans dependent manners, although this concept is still controversial. The cis-acting lncRNAs are restricted to the site or chromosome of synthesis and tend to regulate only a few genes, while the trans-acting lncRNAs diffuse to other chromosomes and regulate wider range of genes (11).

Polycomb respressive complexes (PRCs) are the most common protein partners of lncRNAs that are studied so far. PRCs are known to promote gene repression by modifying the chromatin structure. Intrinsic histone methyltransferases activity maintains gene repression by methylation of specific amino acid on histone tails that result in compaction of chromatin and formation of heterochromatin (12). Several large lncRNAs are known to interact with PRCs (13). One study in 2009 indicated that more than 20% of the lncRNAs interact with PRCs (6). LncRNA transcripts are critical for recruitment of the proteins in some cases where lncRNAs serve as scaffold for protein complexes. HOTAIR is known to scaffold PRC2 and LSD1 that localize to thousands of sites throughout the genome (14).

3.2. Transcriptional regulation

Several lines of evidence suggest that lncRNAs directly regulate gene expression by influencing the activity of promoter enhancers. Kim *et al*. recently found that more than 12,000 neuronal activity-regulated enhancers are transcribed bi-directionally to a class of lncRNA called enhancer RNAs (eRNAs) and that the expression of eRNAs was positively correlated to the mRNA level of nearby protein coding genes (15). Other groups also observed noncoding transcripts expressed from enhancer sites nearby the protein coding genes (16,17). The biological and mechanistic roles of eRNA are not clearly defined yet, although a few were functionally studied (17). Similarly, eRNAs transcribed from AR-regulated genes enhancers are known to respond to AR signaling and they are related to transcription reprogramming (16). However, there is still a possibility that eRNAs could be the potential byproduct of RNA polymerase.

Interestingly, lncRNAs themselves can act as enhancer (18,19). Huang *et al*. recently found that depletion of several lncRNAs located more than 1kb upstream to the protein coding genes resulted in corresponding decrease in neighboring genes including TAL1, Snai1 and Snai2 (Figure 2) (19). The increase in gene expression could be achieved through an RNA-mediated recruitment of transcriptional factors, displacement of transcriptional repressors, recruitment of a basal transcriptional factor or a chromatin-remodeling factor (19). However, the exact mechanism by which lncRNAs act to enhance gene expression is yet to be determined.

3.3. Post-transcriptional regulation

LncRNAs can also regulate gene expression by interfering post-transcription processing of mRNAs. Nuclear body paraspeckles are good examples of how lncRNAs regulate mRNA processing. The cell nucleus is a complex organelle containing many classes of nuclear bodies, such as ribosome biogenesis, transcription, and RNA splicing, that are involved in different nuclear activities (20). Paraspeckles contain at least 3 RNA binding proteins and all of them contain RNA binding domains, which suggest that their function may be related to RNA modification (21). A ~8 Kb nuclear-retained poly(A)+ called CTN-RNA was found to localize to paraspekles and also to distribute throughout the nuclei. CTN-RNA is a counterpart of a protein coding gene, mCAT2. Interestingly, knockdown of CTN-RNA resulted in decrease in mCAT2 mRNA. CTN-RNA is stored in the paraspekles when mCAT2 is not immediately required by the cell. However, when the cell is under stress, CTN-RNA is cleaved to mCAT2 mRNA resulting in increased mCAT2 protein expression (22). More recently, ncRNA MENε/β were found to be involved in paraspeckle assembly (23). Additionally, nuclear-enriched autosomal transcript 1 (NEAT1) lncRNA was also found to be co-localized with the paraspeckles and serve as a structural RNA. The 4 Kb lncRNA contains many self-complementary sequences that could either form an intramolecular structure or make hybrid with each other to create a large scaffold upon which the large nuclear body could build (24). These results suggest that many different lncRNAs can work together to regulate RNA processing (Figure 3).

LncRNAs also regulate mRNA translation by controlling mRNA stability. Nuclear-enriched lncRNA antisense to mouse ubiquitin carboxy-terminal hydrolase L1 (Uchl1) increases UCHL1 protein synthesis at a post-transcriptional level by combining with an embedded SINEB2 (short interspersed nuclear elements) that confers the protein synthesis activation domain and 5' antisense region to specify the target sense mRNA (25). Antisense Uchl1 is a presenter of sense-antisense (S-AS) pair lncRNAs. LncRNAs also suppress mRNA

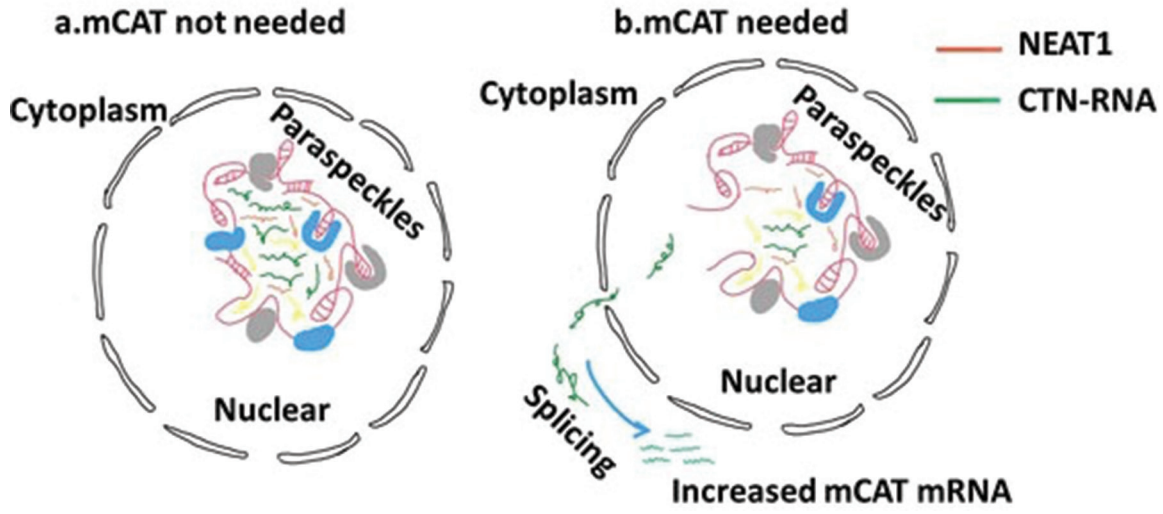

Figure 3. Paraspeckles lncRNA. (a) When cell is not under stress, NEAT1 (red) tether itself and other proteins to form the Paraspeckles which stores RNA in the nuclear including the lncRNA CTN-RNA. (b). When cell is under stress, mCAT is needed. Paraspeckles release the CTN-RNA. CTN-RNA is then transferred to the cytoplasm and spliced to mCAT mRNA followed by increase in mCAT mRNA and protein expression.

Table 1. Oncogenic and suppressive roles of LncRNAs in breast cancer

LncRNA name	Function	Mechanism	Reference
Oncogenic			
H19	Gene silencing Promote anchorage-independent growth	Epigenetic	38,39,40,41
SRA	Expression activator	Transcriptional	54,55
LSINCT5	Promote proliferation	Not known	57
Zfas1	Promote proliferation	Not known	59
LncRNA-Smad7	Anti-apoptosis	Not known	60
LOC554202	Controversy	Not known	62
HOTAIR	Suppress invasion and migration	Epigenetic	68,69,70,71
SOX2OT	Induce SOX2 expression	Epigenetic	73
FAL1	Stabilize BMI1 protein Suppresses p21	Post-transcriptional	75
Tumor suppressive			
GAS5	Induce apoptosis	Transcriptional	79,80,81,82
XIST	Not known	Epigenetic	92,93,94

translation via different mechanisms such as the case of lncRNA-p21 (26,27). This transcript binds to CTNNB1 and JUNB mRNA and represses the translation of mRNA by displacing the polysome or inducing ribosome drop-off that results from the base-pair interaction between lncRNA and target mRNAs (26).

Several recent studies have highlighted a lncRNA-mRNA interaction which is very similar to the miRNA regulation of mRNA (28, 29). LncRNA that possess Alu elements is able to mediate mRNA decay by binding to the 3'UTR (29). Alu elements, which constitute 10% of all the human DNA sequence, are the most frequently observed repeats in human genome. Base pairing of Alu containing lncRNA with mRNA 3' UTR creates a STAU1, RNA degradation protein, binding site and leads to mRNA decay (30). Similar to miRNAs, one lncRNA can bind to a subset of mRNA and one mRNA can be targeted by multiple lncRNAs. LncRNAs are also reported to bind (bind to) miRNA and work as a miRNA sponge. LncRNA-ATB suppresses miR200 family and LncRNA MD1 suppresses miR-133 and miR-135 in this manner (31,32). Therefore, this RNA-RNA interaction is considered to be another level of post transcription regulation of gene expression by lncRNAs.

As described above, lncRNAs can regulate gene expression at multiple stages by a variety of mechanisms, and it is becoming clear that aberrant expression of such lncRNAs can significantly contribute to tumor initiation and progression. In the following sections, we summarize the roles of lncRNAs by particularly focusing on breast cancer.

4. ONCOGENIC LNCRNAS IN BREAST CANCER

4.1. Proliferation and Apoptosis

4.1.1. H19

H19 is one of the well-characterized imprinting lncRNAs. It is a 2.3.Kb transcript encoded by the maternal allele and is capable of silencing the IGF2 gene in the

same allele (33). H19 is known to be involved in controlling embryonic growth and gene imprinting regulation (34, 35). The network of imprinted genes including IGF2 is under the control of H19 during embryogenesis (36). MBD1, a gene that maintains repressive marker (H3K9me3 modification of histone), is recruited and tethered by H19 to the target genes and that suppresses the gene expression (37). H19 is also known to be abnormally overexpressed in cells of higher tumorigenic capacity and it has been considered as an oncogenic RNA in breast epithelial cells (38-41). In breast cancer cells, H19 was up-regulated in S-phase and promoter was found to be activated by E2F1 (42). H19 is also reported to be regulated by c-myc which is a widely dysregulated transcription factor in cancer of epithelial origin including breast cancer. C-myc binds to the conserved E-box on the promoter of H19 and facilitates transcriptional initiation of H19, whereas c-Myc down-regulates the IGF expression by binding to the E-box on the first intron (43). However, the mechanism by which C-myc distinguishes these two alleles is not known. Previous works (work) showed that INTRODUCTION of H19 into several tumor cell lines caused suppression of anchorage–independent growth, and therefore, H19 was considered as a tumor suppressor (44,45). However, in another study, knockdown of H19 resulted in decrease in clonogenicity of cancer cells and suppression of anchorage-independent growth in breast cancer cell lines, MDA-MB231, SKBR3, T47D and A459 (40). Although the exact function of H19 in tumor cells is still unclear, it appears to play a pivotal role in the tumorigenic phenotype in breast cancer. The mechanism of H19 action is thought to be translational regulation; however, this needs further verification.

Interestingly, H19 was reported to antagonize let-7 microRNA which negatively regulates self-renewal of cancer stem-like cells and tumorigenicity in breast cancer (46,47). In this study, H19 acted as a molecular sponge of Let-7 and affected the expression of endogenous let-7 targets. H19 is also known to be the precursor of miR675 which is an oncogenic miRNA and suppresses pRB expression (48,49).

4.1.2. SRA

Steroid receptor RNA activator (SRA) is the first lncRNA discovered to function independently of epigenetic or catalytic mechanism (50). However, SRA also codes a protein (51). SRA selectively responds to hormone receptors and mediates transactivation of steroid receptor-dependent genes (52). Upon steroid stimulation, the nuclear receptor directs the assembly and stabilization of a transcription complex at the promoter of a target gene. SRA also interacts with other proteins such as SRC-1 to form a large complex which selectively enhances transcription of steroid response genes (53).

SRA has been reported to be upregulated in breast cancers, suggesting a potential oncogenic role. The transgenic MMTV-SRA mouse bears a human non-coding SRA sequence under the control of the mouse mammary tumor virus (MMTV) promoter (50,54,55). In this mice model, SRA is expressed only in female mammary gland cells, and the mice showed aberrant mammary gland formation where multilayers of epithelial cells, ductal luminal hyperplasia and lymphocyte infiltration were observed (55). Overexpression of SRA also promoted cell proliferation and differentiation (56). Although these results suggest the potential oncogenic role of SRA, the transgenic mice exhibited normal life span, indicating that the overexpression of SRA is not sufficient to induce full blown breast cancer.

4.1.3. LSINCT5

LSINCT5 is a 2.6.KB stress-induced antisense lncRNA. Normally, LSINCT5 localizes to the nucleus and it is potentially transcribed by RNA polymerase III instead of RNA polymerase II (57,58). LSINCT5 is overexpressed in various breast cancer cell lines and tumor tissues compared to normal breast epithelial cells. Knockdown of LSINCT5 resulted in decrease in cell proliferation in breast tumor cell lines. Smith *et al* found 95 potential targets of LSINCT5 by Affymetrix array, and that two genes, lncRNA NEAT1 and protein coding gene PSPC1, were most significantly regulated by LSINCT5 (57). However, these targets were not experimentally validated and the mechanism of function is yet to be known. Nevertheless, LSINCT5 is considered to be a potential oncogenic lncRNA due to its effect on cellular proliferation which warrants further study of this otherwise intresting lncRNA.

4.1.4. Zfas1

Zfas1 is the antisense to the 5'end of the protein-coding gene, Znfx1, and host snoRNAs . Previously, Zfas1 was only considered as a vehicle to generate snoRNAs. However, it was later found to localize within the ducts and alveoli of mammary gland and appeared to be involved in mammary gland development as it was found to be differentially expressed between pregnancy and lactation. Knockdown of Zfas1 in breast epithelial cells resulted in increased cell proliferation, and Zfas1 expression in breast cancer cells is reduced relative to normal breast tissues (59). Therefore, Zfas1 is a novel and potential breast cancer suppressor which requires future research to clarify its specific function and mechanism in breast tumorigenesis.

4.1.5. LncRNA-Smad7

LncRNA-Smad is recently identified as located adjacent to the mouse Smad7 gene. The expression of LncRNA-Smad7 is induced by TGF-beta in all mammary gland epithelial cells and breast cancer cell lines. Suppression of this lncRNA neutralized the anti-apoptosis function of TGF-β. In contrast, ectopic expression of LncRNA-Smad7 rescued apoptosis induced by a TGF-β receptor inhibitor. But the contribution of this lncRNA

appears to be restricted to apoptosis since knockdown of it didn't affect TGF-β-induced epithelial to mesenchymal transition, phosphorylation of Smad2 or expression of the Smad7 gene (60). This finding suggests a tumorigenic role of this lncRNA, although more detailed mechanism needs to be further clarified.

4.1.6. LOC554202

LncRNA, LOC554202, is known as the host of miR31 (61). Shi *et al*. found that LOC554202 expression was significantly overexpressed in breast cancer tissues and cell lines compared to normal breast tissues (62). Knockdown of LOC554202 decreased breast cancer cell proliferation, induced apoptosis and inhibited migration/invasion *in vitro* and tumorgenesis *in vivo*. However, another study showed that the LOC554202 expression was suppressed in triple negative breast cancer and that promoter hyper-methylation was the major mechanism of silencing this lncRNA (61). Therefore, the role of LOC554202 in breast tumor remains controversial.

4.2. Invasion and metastasis

4.2.1. HOTAIR

HOX transcript antisense RNA (HOTAIR) is a 2.2.Kb transcript derived from the HOXC gene cluster (10). HOTAIR is considered to be an epigenetic regulator and its function and mechanism are relatively well studied. It is the first lncRNA found to work in trans-acting manner (10). The 5' end of HOTAIR is known to interact with the PRC2 complex, and the 3' end of this RNA is capable of binding to the histone demethylase LSD1 (63, 64). HOTAIR guides and also serve as a scaffold for PRC2 and LSD1 at target genes (65). An example is that HOTAIR represses transcription in trans across 40Kb of the HOXD locus by recruiting the PRC2 complex which maintains the histone H3 lysine-27 trimethylation of the locus (10,65,66).

The HOX genes contain both tumor suppressive genes and oncogenic genes in breast cancer (67). There are numerous lncRNAs transcribed from this locus; however, their biochemical functions are not well characterized. HOTAIR is one of the oncogenic lncRNAs and may be an only example of a global regulatory phenomenon. Gupta *et al*. found that HOTAIR expression is upregulated from hundreds to nearly two thousands fold in metastatic breast cancer tissues, whereas HOTAIR expression is often high but heterogeneous in primary breast cancer (68). The expression of HOTAIR in primary breast cancer is a powerful predictor of metastasis and survival (68,69). Furthermore, ectopic expression of HOTAIR was shown to enhance colony formation in soft agar and increase invasion through matrigel. The ectopic expression also modestly increased tumor growth and promoted spontaneous lung metastasis as well as lung colonization *in vivo*. Depletion of HOTAIR in breast cancer cells significantly decreased matrix invasiveness. Mechanistically, ectopic expression of HOTAIR is known to induce localization of PRC2 and H3K27me3 on 854 genes that are mostly down-regulated by HOTAIR. The PRC2 expression pattern in HOTAIR-overexpressed cells resembles the pattern of embryonic fibroblast expression, suggesting that HOTAIR reprograms the PRC binding pattern in breast cancer to that of embryonic fibroblast (68). These findings indicate that HOTAIR has an important role in cancer epigenome and could be a potential biomarker and a therapeutic target for breast cancer.

Chisholm *et al*. reported that HOTAIR expression is highly correlated with EZH2, which is the subunit of PRC2, in breast tumor tissues. The depletion of EZH2 and PRC2 was shown to be significantly increased in metastatic lesions compared to the paired primary breast tumor tissues. In addition, the correlation of expression of these two genes was found to be related to worse outcome in patients (70). Another study of clinical breast tumor samples showed that the level of genomic DNA methylation is positively correlated to HOTAIR, suggesting that the HOTAIR expression may be regulated by intergenic DNA methylation in breast cancer (71).

4.3. Cancer stemness

4.3.1. SOX2OT

The SOX2 gene coding sequence lies within the intron of SOX2 overlapping transcript (SOX2OT) which is a long multi-exon lncRNA (72). Expression of SOX2 and SOX2OT is positively correlated in breast cancer, and SOX2OT is differentially expressed between ER positive and ER negative breast cancer (73). Both SOX2 and SOX2OT are upregulated in cancer stem like cells, and SOX2 is one of key genes in maintaining pluriopotency (74). Furthermore, ectopic expression of SOX2OT leads to dramatic increase in SOX2 expression and subsequent increase in breast cancer anchorage-independent growth (73). These results indicate the potential oncogenic role of SOX2OT in breast cancer by inducing or maintaining the expression of SOX2.

4.3.2. FAL1

LncRNA focally amplified on chromosome 1 (FAL1) was identified by Zhang's group through the global genomic analysis, and it is a potential oncogenic lncRNA in breast cancer. Knockdown of FAL1 significantly reduced the clonogenicity and inhibited cell proliferation and anchorage-independent growth of breast cancer cells. The knockdown of FAL1 also suppressed growth of subcutaneous tumor formed by MDA-MB-231 cell lines (75). Interestingly, FAL1 expression is associated with BMI1 which is a well-known stemness associated factor of cancer cells (76). Later, FAL1 was found to interact directly with BMI1 by RNA pull down assay. This study also revealed an 116nt region which is the major binding domain for BMI1. Moreover, the knockdown of FAL1 significantly decreased the protein level but

not the mRNA level of BMI1, suggesting that FAL1 regulates BMI1 at a post translational level by stabilizing the BMI1 protein. FAL1 was also found to regulate the transcription of a large set of genes that are important for cellular proliferation, death and survival as well as cellular movement and protein degradation. One of these genes is p21 which functions as a key tumor suppressor in many cancer types (75).

5. TUMOR SUPPRESSIVE LNCRNAS IN BREAST CANCER

5.1. Proliferation and Apoptosis

5.1.1. GAS5

The growth arrest-specific 5(GAS5) is a non-coding RNA which plays a critical role in controlling mammalian cell apoptosis as well as proliferation (77). GAS5 was originally identified in mouse NIH 3T3 cells and it was specifically expressed when cells were under growth arrest (78). The GAS5 gene hosts several small nucleoli RNAs and is subject to complex post-transcriptional processing (77).

The expression of GAS5 was found to be significantly down-regulated in breast cancer cells compared to unaffected normal adjacent cells. Williams's *et al*. have recently reported that overexpression of GAS5 in MCF10A and MCF7 cells enhanced apoptosis that was mediated by UV irradiation and cisplatin (79). Consistent with their observation, Ozgur *et al*. also reported that GAS5 was up regulated in MCF7 cells during genotoxic stress-induced apoptosis (80). In some cases, ectopic expression of GAS5 alone causes growth arrest and induces apoptosis in breast cell lines. Furthermore, cells under growth arrest due to a lack of nutrients or growth factors were found to express a high level of GAS5 which sensitize cells to apoptosis (81). Kino *et al*. found that GAS5 bound to the DNA binding domain of the glucocorticoid receptor (GR) and competing with glucocorticoid response element (GRE) which is a regulatory sequence in the genome (77). Thus, GREs are sequestered and glucocorticoid-mediated induction of several responsive genes are suppressed including cellular inhibitor of apoptosis 2 (cIAP2) (77). GAS5 was also found to be regulated by miR21 which is a well-studied oncogenic micro RNA (82). MiR21 regulates numerous genes involved in cell growth and apoptosis, and the tumor suppressor, PTEN, is its well validated target (83). Using a qRT-PCR based array, Zhang *et al*. found that GAS5 was significantly reduced in MCF7 cells with miR21 knockdown compared to the control MCF7 cells (82). In fact, a complementary region with miR21 was identified in the GAS5 sequence. GAS5 expression was also found to be negatively correlated with miR21 expression in clinical specimens of breast cancer. Interestingly, when GAS5 was knocked-down, the miR21 expression elevated and ectopic expression of GAS5 decreased miR21 expression, suggesting reciprocal suppression of miR21 by GAS5 (82). This suppression was regulated by RISC which implies that factors involved in micro RNA biogenesis or processing also regulates microRNA and lncRNA expression (82,84). These studies indicated that GAS5 is a potential tumor suppressor which impacts the stress-induced cell growth inhibition, apoptosis and tumor invasion.

5.2. Invasion and metastasis

5.2.1. XIST

XIST is critical for the X chromosome inactivation in embryogenic development. The 17Kb long lncRNA spreads along the X chromosome and recruits PRC to maintain silencing of the X chromosome (85). A recent study by Jeon *et al.* showed that YY1 guides XIST loading and spreading during the initial phases of X chromosome inactivation . This dosage compensation resulted in silencing of more than 1000 genes on one of the X chromosome (86).

The role of XIST in breast cancer has been intensively studied but remains controversial and unclear. The Livingston group showed that BRCA1, a breast tumor suppressor, supported XIST RNA concentration on the inactive X chromosome. BRCA1 was found to co-localize with inactive X chromosome, and plays a critical role in maintaining markers of X chromosome inactivation (87). This observation suggests that loss of BRCA1 in female cells may lead to destabilization of X chromosome. However, Xiao *et al*. found that XIST functions independently of BRCA1 in X inactivation (88). According to results of Livingston group, the loss of BRCA1 in female cells could reactivate genes on the inactive X chromosome due to loss of XIST (87); however, the reason of this apparent controversy is not clear. They also found that BRCA1 was not enriched on XIST RNA-coated chromatin of inactive X chromosome (88). To respond to these findings, Livingston group showed further evidences of BRCA1's communication with inactive X chromosome (89). The interaction between BRCA1 and XIST remains an issue of debate; however, it is of great importance to understand the underlying mechanism by which XIST interact with BRCA1 (90,91).

In another study, the expression of XIST was found to differ between subtypes of breast cancer and also related to BRCA1 status. Only the XIST, which was expressed from the inactive X chromosome, was regulated by BRCA1 (92). However, XIST can also be abnormally expressed from an active X chromosome. Although most breast tumor cell lines lost the inactive X chromosome and gained one more active X chromosome, whether loss of XIST is leading to the loss of inactivated X chromosome or loss of XIST leads to the loss of inactive X chromosome is not clear (93). These results indicate that the mechanism of XIST expression in breast cancer cells may be different from that of the normal cells.

Andrea *et al*. recently reported that XIST misbehaved particularly in basal like breast cancer (BLC) cells. In this study, 80% of the samples showed X chromosome abnormality, and duplication of the active X chromosome and loss of X inactivation center were most frequently seen in these samples. However, there were only 2 out 20 samples that lost their inactive X chromosome in non-BLC patients. This misbehavior of XIST is not related to BRCA1 because most samples retained wild type BRCA. Surprisingly, the expressions of X linked genes were not globally upregulated in those BLC samples (94). There were only few loci that escaped X chromosome inactivation in the samples that lost XIST. These studies suggest that X chromosome abnormalities may contribute to the BLC formation; although the mechanism and effect of these abnormalities are yet to be understood. A future study is needed to clarify the relation of XIST and X chromosome abnormalities to understand the pathological role of this lncRNA in breast cancer.

6. FUTURE DIRECTION

Due to the rapid development of high-throughput sequencing technique and genomic profiling along with the powerful bioinformatics tools, significant amount of information about lncRNAs have been emerged in the last decade (7). Comprehensive sequencing analysis has been a useful tool to identify novel lncRNAs and to discover new roles of lncRNAs by providing a framework for the current lncRNA studies. Maruyama *et al*. recently analyzed transcriptome from normal and breast cancer epithelial cells by specifically focusing on the distribution of sense and antisense lncRNA between normal and cancer (95). Many of the differentially expressed antisense transcripts likely represent lncRNAs. Certain genes only generate antisense transcripts in normal or cancer and they are involved in important physiological function such as tumor cell metabolism. Zhao *et al*. comprehensively characterized the lncRNAs between different breast cancer subtypes. They observed 20 lncRNA genes that are significantly correlated with breast cancer subtypes and 14 lncRNAs significantly correlated with breast cancer grades. They also identified 3 lncRNAs (LINC00324, PTPRG-AS1 and SNHG17) that were significantly correlated with clinical outcomes of patients (96). Su *et al*. also analyzed lncRNAs in breast cancer subtypes and classified lncRNAs into 4 subtypes with different prognoses (97). Furthermore, Reiche *et al*. analyzed microarray data of mRNA from clinical specimen and identified 19,000 transcripts that were significantly and differentially expressed between normal and cancer breast tissue (98). Interestingly, they found that more than half of these transcripts were lncRNAs. A number of these genes have also been identified in relation to cancer associated protein coding genes. Although their biological function in breast cancer is not yet clear, both computational and experimental analysis should address the molecular mechanism of lncRNAs.

Breast cancer is known to be driven by many different oncogenic pathways, and there are multiple steps that are involved in cancer progression and invasion (99). Therefore, screening based on functional assays could also identify novel lncRNAs that are involved in tumor progression. In addition, it is well established that tumor microenvironmental cells play pivotal roles in regulating breast cancer progression (100). However, the roles of lncRNA in these cells are not well explored. It should be noted that lncRNAs are also secreted in exosome by cancer cells, which indicates that lncRNA may play a critical role in the communication of cancer cells and microenvironmental cells and metastasis niche formation (101,102).

LncRNAs represent a novel and rarely characterized components of the cancer cells, and therefore, there is a great potential to develop biomarkers using these RNAs for clinical use (103, 104). Future development of lncRNA-based therapy is not on sight right now; however, the recent breakthrough of CRISPR system for genome editing *in vivo* holds great promise for targeting lncRNAs for cancer treatment (105-107).

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